FEBS Letters 587 (2013) 3360–3364

journal homepage: www.FEBSLetters.org

Aminoacylation of tRNA 2′- or 3′-hydroxyl by phosphoseryl- and pyrrolysyl-tRNA synthetases

Markus Englert [a], Sarath Moses [a], Michael Hohn [a], Jiqiang Ling [a], Patrick O'Donoghue [a], Dieter Söll [a,b,*]

[a] Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520-8114, USA
[b] Department of Chemistry, Yale University, New Haven, CT 06520-8114, USA

ARTICLE INFO

Article history:
Received 13 August 2013
Revised 22 August 2013
Accepted 23 August 2013
Available online 8 September 2013

Edited by Michael Ibba

Keywords:
Protein synthesis
tRNA esterification
Amino acid

ABSTRACT

Class I and II aminoacyl-tRNA synthetases (AARSs) attach amino acids to the 2′- and 3′-OH of the tRNA terminal adenosine, respectively. One exception is phenylalanyl-tRNA synthetase (PheRS), which belongs to Class II but attaches phenylalanine to the 2′-OH. Here we show that two Class II AARSs, *O*-phosphoseryl- (SepRS) and pyrrolysyl-tRNA (PylRS) synthetases, aminoacylate the 2′- and 3′-OH, respectively. Structure-based-phylogenetic analysis reveals that SepRS is more closely related to PheRS than PylRS, suggesting that the idiosyncratic feature of 2′-OH acylation evolved after the split between PheRS and PylRS. Our work completes the understanding of tRNA aminoacylation positions for the 22 natural AARSs.

1. Introduction

Aminoacyl-tRNA synthetases (AARSs) are essential enzymes that catalyze the attachment of amino acids to corresponding tRNAs [1]. The resulting aminoacyl-tRNAs (AA-tRNAs) are transported by the elongation factor (EF-Tu in bacteria and EF1A in archaea and eukaryotes) to the ribosome as building blocks for protein synthesis [2,3]. AARSs catalyze formation of AA-tRNAs in two steps at the same active site: activation of the amino acid with ATP to form an aminoacyl-adenylate (AA-AMP), and transfer of the amino acid moiety to the 2′- or 3′-hydroxyl group (OH) of the tRNA terminal adenosine (A_{76}) [1,4]. Based on the active site structure, AARSs are grouped into two independently evolved classes [5]. Class I AARSs all attach amino acids to the 2′-OH of A_{76}, whereas most Class II enzymes aminoacylate the 3′-OH except asparaginyl- (AsnRS) and phenylalanyl-tRNA (PheRS) synthetases that prefer the 2′-OH [4,6,7].

In solution, the 2′- or 3′-linked amino acid spontaneously transacylates to the neighboring OH at high rates [8], resulting in a mixture of 2′- and 3′-linked AA-tRNA isomers. EF-Tu stabilizes the 3′-isomer, which is preferred by the ribosome during peptide bond formation [9]. The vicinal hydroxyl group plays critical roles in catalyzing peptide bond formation on the ribosome, and hydrolyzing (editing) misacylated tRNAs by several AARSs and *trans*-editing factors [10–13].

SepRS and PylRS belong to the Class IIc subfamily and are evolutionary related to PheRS [14–17]. Except in mitochondria, PheRS forms $(\alpha\beta)_2$ heterotetramers [18,19]. SepRS is a α_4 homotetramer, and PylRS forms α_2 homodimers [15,17]. SepRS aminoacylates *O*-phosphoserine (Sep) onto $tRNA^{Cys}$ in several methanogenic archaea, and the resulting Sep-$tRNA^{Cys}$ is then converted to Cys-$tRNA^{Cys}$ by Sep-tRNA:Cys-tRNA synthase [14]. In methanogens and a few anaerobic bacteria, PylRS attaches pyrrolysine (Pyl) onto $tRNA^{Pyl}$ with a CUA anticodon, which decodes in-frame UAG codons [16]. PylRS has been extensively engineered for genetic incorporation of non-canonical amino acids [20–22]. SepRS has also been used to expand the genetic code of *Escherichia coli* with Sep [23]. Here we show that SepRS more closely resembles PheRS and attaches Sep onto the 2′-OH of A_{76}, whereas the more distantly related PylRS aminoacylates the 3′-OH. Structural modeling reveals that the aminoacylation active site of SepRS superimposes well with that of PheRS and that 2′-OH aminoacylation by PheRS and SepRS is presumably an intrinsic feature determined by the active site architecture.

2. Materials and methods

2.1. Preparation of tRNA transcripts with 3′-terminal adenosine, 2′-deoxyadenosine or 3′-deoxyadenosine

In vitro T7 RNA polymerase runoff transcripts of *Methanocaldococcus jannaschii* $tRNA^{Cys}$ and *Methanosarcina barkeri* $tRNA^{Pyl}$ were prepared from linearized plasmids as described previously [24]. To

* Corresponding author at: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520-8114, USA. Fax: +1 203 432 6202.
E-mail address: dieter.soll@yale.edu (D. Söll).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.08.037

generate tRNA transcripts with a 3′-CC_{75} end, a *Fok*I restriction site was placed in appropriate distance to the 3′ end of each tRNA gene sequence. Transcripts were purified on a denaturing 12% polyacrylamide gel, recovered by passive elution in 1 M sodium acetate (pH 6.0), ethanol precipitated, resuspended in double distilled water, desalted on Sephadex G25 Microspin columns (Amersham), and refolded as described [25]. The addition of the 76th nucleotide was achieved by using *E. coli* terminal tRNA nucleotidyl transferase under the following conditions. 20 μM tRNA CC_{75} transcript was mixed with 2 mM ATP, 2′-dATP, or 3′-dATP in a reaction buffer containing 50 mM glycin–NaOH (pH 9.0), 10 mM $MgCl_2$, 7 mM 2-mercaptoethanol, and 1 μM terminal tRNA nucleotidyl transferase. After 1 h of incubation at 37 °C, tRNA was phenol and chloroform extracted, precipitated, resuspended in double distilled H_2O and desalted on a Sephadex G25 Microspin column. The addition of the modified 3′-terminal adenosine was checked on a 8% denaturing polyacrylamide gel.

2.2. Aminoacylation of tRNA transcripts

The standard reaction mixture (50 μl) contained 50 mM Hepes–KOH, pH 7.0, 50 mM NaCl, 20 mM $MgCl_2$, 5 mM dithiothreitol, 10 mM ATP, 200 μM [^{14}C]phosphoserine (55 mCi/mmol), 10 μM tRNA transcript, and 10 μM purified recombinant *M. maripaludis* SepRS. Radioactive aminoacyl-tRNAs synthesized after 2–45 min were quantified in 9-μl aliquots as described previously [25].

2.3. Acid urea gel electrophoresis of aminoacyl-tRNA

The aminoacylation of the modified $tRNA^{Pyl}$ transcripts can be assayed by the different mobility shift of the charged and uncharged species on an acid urea gel [16]. The aminoacylation reaction was performed for 90 min at 37 °C in 100 mM Na–Hepes (pH 7.2), 60 mM NaCl, 25 mM $MgCl_2$, 5 mM ATP, 5 mM DTT, 1 mM pyrrolysine, 2 μM of 5′-[γ-^{32}P]ATP labeled modified $tRNA^{Pyl}$ transcript, and 5 μM *M. barkeri* and 10 μM *Desulfitobacterium hafniense* PylRS respectively. All reactions were started by addition of the amino acid. The reactions were stopped with 1 volume of 0.3 M sodium acetate (pH 4.5)/10 mM ethylenediaminetetraacetic acid (EDTA). After phenol/chloroform extraction and ethanol precipitation, the aminoacyl-tRNAs were dissolved in 1× loading buffer [7 M urea/0.3 M sodium acetate (pH 4.5)/10 mM EDTA/0.1% bromophenol blue/0.1% xylene cyanol] and were loaded onto a 6.5% polyacrylamide gel (50 × 20 cm, 0.4 mm thick) containing 7 M urea and 0.1 M sodium acetate (pH 5.0). The gel was run at 4 °C, 500 V, in 0.1 M sodium acetate (pH 5.0) for 18 h. Charged and uncharged tRNAs were exposed to phosphoimager plates (FujiFilms), visualized using a Molecular Dynamics Storm 860 scanner (Amersham) [16].

2.4. Structure-based phylogenetic analysis

Protein structures were downloaded from the protein databank or the SCOP database [26] and aligned using Multiseq 2.0 [27]. The tree was calculated from the structural similarity metric QH [28]. The tree was drawn based on the QH distance matrix computed in Multiseq 2.0 using Phylip 3.66 Neighbor and Drawtree programs [29].

3. Results and discussion

To characterize the aminoacylation mechanisms of SepRS and PylRS, we prepared transcripts of $tRNA^{Cys}$ and $tRNA^{Pyl}$ in which the 3′-terminal adenosine was exchanged with 2′- or 3′-deoxyadenosine, and used them as substrates for aminoacylation by SepRS or PylRS. *M. maripaludis* SepRS charged $tRNA^{Cys}$ with a 3′-deoxyadenosine, but not $tRNA^{Cys}$ without A_{76} or with a 2′-deoxyadensosine (Fig. 1), suggesting that SepRS attaches Sep onto the 2′-OH of A_{76}. The charging efficiency of $tRNA^{Cys}$ ending with 2′-deoxyadenosine was three times lower compared to transcripts terminally labeled with adenosine, indicating that the 3′-OH group promotes aminoacylation. In contrast, both *M. barkeri* and *D. hafniense* PylRS attaches Pyl only to tRNA transcripts with a 3′-OH group at A_{76}, whereas no aminoacylation at the 2′-OH group was detected (Fig. 2). PylRS thus behaves like a normal Class II AARS charging the 3′-OH of A_{76}, and SepRS resembles PheRS in aminoacylation of the 2′-OH (Fig. 2).

Given the biochemical data, the evolution of 2′-OH can be interpreted in reference to the phylogenetic tree of the class II AARS family (Fig. 3). This protein family is distantly related, and some class II AARSs share only 5% sequence identity. The AARS family tree is, therefore, based on a structural overlap of the available X-ray structures of class II AARSs, and the branch lengths are computed from the structural similarity between the AARS catalytic core domains (as in [28]). The tree indicates that PylRS diverged from a PheRS ancestor perhaps with a 3′-OH preference given that PylRS prefers 3′-OH as do most class II AARSs. Following the divergence of PylRS and PheRS, the PheRS enzyme acquired a 2′-OH preference (Fig 3. red arrow), which was inherited by SepRS after its later divergence from PheRS. PylRS was predicted [30] to prefer the 2′-OH, based on the close relationship between PylRS, PheRS, and SepRS. The biochemical and phylogenetic data presented here indicate that the 2′-OH preference evolved only after PylRS emerged, which underscores the fact that it is not evident from structure or sequence data why PheRS and SepRS differ in aminoacylation position.

The distinction of AARSs into two classes correlates remarkably with two different modes of amino acid addition to the tRNA. The idiosyncratic acylation position of PheRS is due to a unique mechanism by which it binds the CCA end of the tRNA (Fig. 4A) [31], which allows it to acylate the 2′-OH group of A_{76}. The co-crystal structure of *Thermus thermophilus* PheRS complexed with $tRNA^{Phe}$ and a Phe-AMP analog reveals a tRNA binding state before amino acid transfer (Fig. 4). Neither the 2′- or 3′-OH in the structure is suitably-positioned to attack the phosphodiester bond of Phe-AMP. However, the PheRS:$tRNA^{Phe}$ complex structure (without Phe or Phe-AMP analog) shows that the A_{76} partially overlaps with the amino acid binding pocket [18], suggesting that substantial

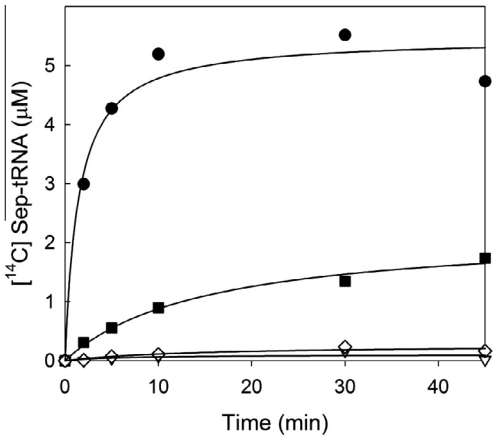

Fig. 1. Site of amino acid attachment in *M. maripaludis* SepRS. Time course of aminoacylation of different $tRNA^{Cys}$ species with Sep using *M. maripaludis* SepRS is shown. The tRNAs were modified at the 3′ termini as follows: tRNA lacking the 3′-terminal adenosine (▽), terminus reconstructed with 3′-deoxyadenosine (■), 2′-deoxyadenosine (◇), and adenosine (●).

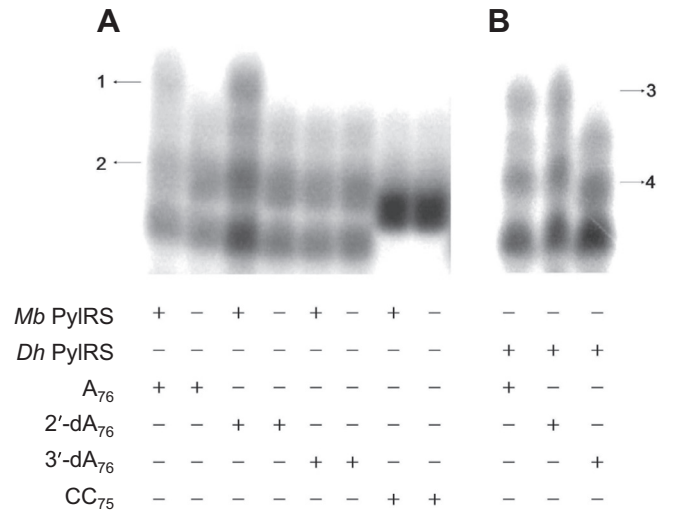

Fig. 2. Analysis of the 3′ terminal adenosine modified *M. barkeri* Fusaro $tRNA^{Pyl}$ transcripts charged with pyrrolysine (Pyl) in the presence (+) and absence (−) *M. barkeri* Fusaro PylRS enzyme. The resulting tRNA products were loaded onto an acid gel and the samples were later exposed to a phosphoimager plate. The positions of $tRNA^{Pyl}$ and Pyl-$tRNA^{Pyl}$ are indicated. The lower band is the unmodified tRNA transcripts with a 3′-CC_{75} end. 1 and 3 indicates the position of Pyl-$tRNA^{Pyl}$, while 2 and 4 indicates uncharged $tRNA^{Pyl}$. *Mb*, *Methanosarcina barkeri*; *Dh*, *Desulfitobacterium hafniense*.

movement of A_{76} occurs upon Phe binding and Phe-AMP formation. Future computational and structural studies (e.g., using non-hydrolyzable Phe-tRNA) might help address the question as to how the 2′-OH of $tRNA^{Phe}$ attack the carbonyl group of Phe.

The SepRS active site superimposes well onto PheRS, and Sep adopts a similar orientation as Phe at the amino acid binding pocket (Fig. 4B). The 3′-end of $tRNA^{Sep}$ is disordered in the SepRS:$tRNA^{Sep}$ complex structure [32], therefore the binding mode of A_{76} at the active site is unclear at a molecular level. It is suggested that features in the AARS, but not the tRNA, determines the site of aminoacylation at A_{76} [4]. Not surprisingly, SepRS attaches amino acids to the 2′-OH as PheRS due to the structural similarity.

Structures of PylRS complexed with $tRNA^{Pyl}$ or Pyl have been solved separately [33,34]. Modeling results reveal that the 3′-OH of A_{76} appears to be 2 Å closer to the carboxyl group of Sep compared with the 2′-OH (Fig. 4C). This is consistent with our observation that 3′-OH is the target of aminoacylation in PylRS, although the molecular mechanism of esterification awaits elucidation.

One puzzling question is why some AARSs aminoacylate the 2′-OH but others choose the 3′-OH. One possible explanation is the requirement for post-transfer editing, a process many AARSs use to hydrolyze misacylated tRNAs [35,36]. PheRS is able to hydrolyze Tyr-$tRNA^{Phe}$ with a fluorine substitution at the 3′-OH of A_{76}, albeit at a lower efficiency [12]. This suggests that the 2′-linked isomer is a substrate for the PheRS editing site, which is separated from the aminoacylation active site by about 40 Å [37]. In the Class I LeuRS that aminoacylates the 2′-OH, the 2′-linked, but not the 3′-linked AA-tRNA analog inhibits editing [38]. In YbaK, a trans-editor factor that hydrolyzes Cys-$tRNA^{Pro}$ produced by the Class II ProRS (aminoacylates the 3′-OH), only the 3′-linked Cys-tRNA inhibits editing [13]. Misacylated tRNA may directly translocate from the active site to the editing site without leaving the AARS or being exposed to solvent to allow transacylation [35]. In this scenario it becomes important that the aminoacylation position on the tRNA is the same as the editing position, so that the mismatched AA-tRNA can directly enter the editing site for hydrolysis. Such a model needs to be tested in future studies.

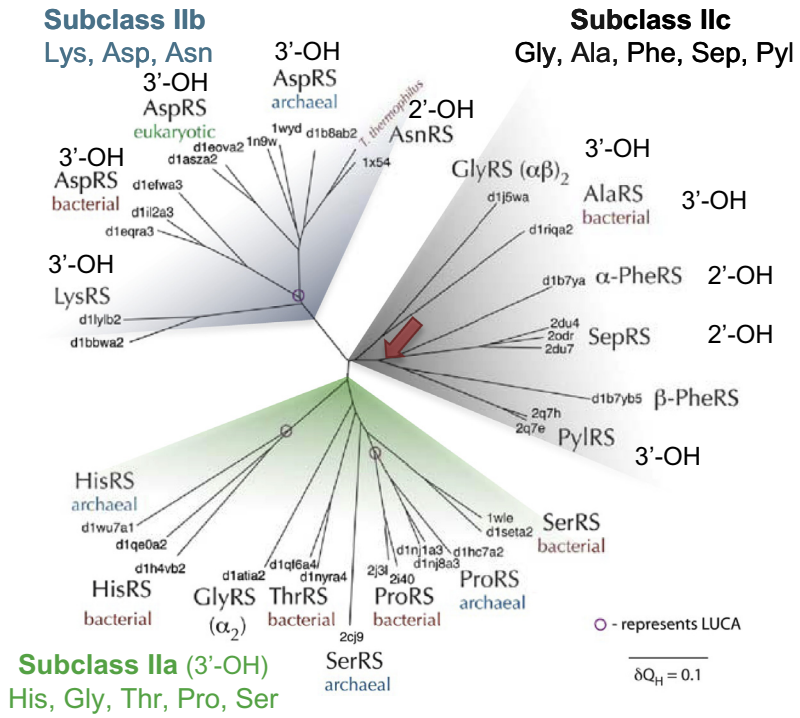

Fig. 3. The structure-based phylogenetic tree (calculated as in [28]) shows the evolutionary relationships between the class II AARS family and subclasses. The table of each aminoacyl-tRNA synthetase with its preference for the attachment of the amino acid to the 2′-OH or 3′-OH as compiled earlier [4] is combined with the experimentally determined specificity of PylRS and SepRS. Every member of the family directs aminoacylation to the 3′-OH of its cognate tRNA. PheRS, AsnRS, and SepRS are the only exceptions and preferentially aminoacylate the 2′-OH. The earliest point in evolution when the switch to 2′-OH preference occurred is indicated (red arrow).

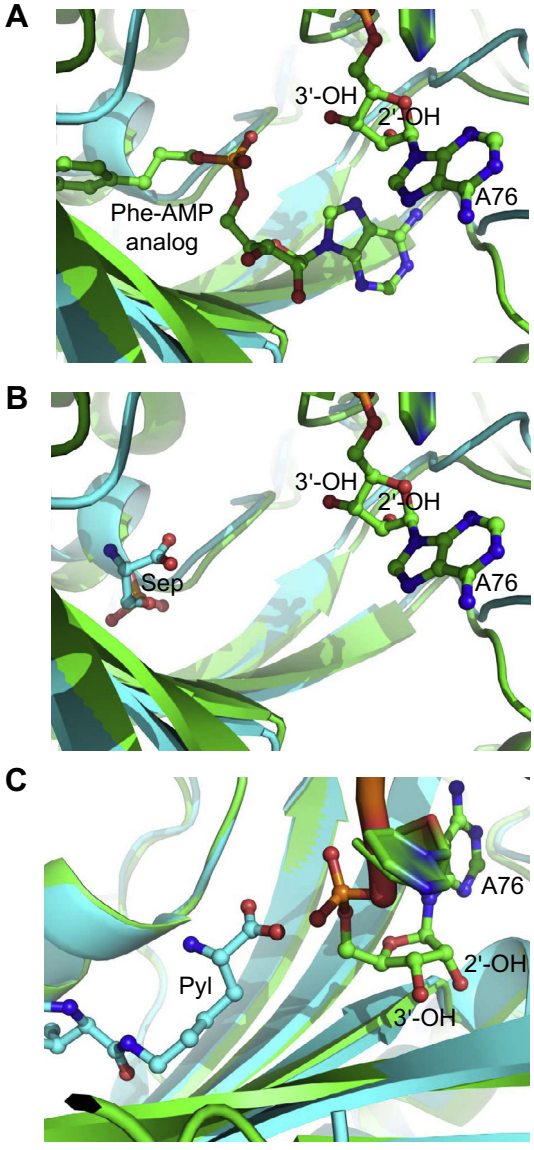

Fig. 4. Structural modeling of PheRS, SepRS, and PylRS aminoacylation active sites. (A and B) *A. fulgidus* SepRS structure complexed with Sep (cyan, PDB: 2DU3) is superimposed onto the structure of *T. thermophilus* PheRS:$tRNA^{Phe}$:Phe-AMP analog complex (green, PDB: 2IY5). (C) The structure of *Methanosarcina mazei* PylRS complexed with Pyl (cyan, PDB: 2ZCE) is superimposed onto the structure of *D. hafniense* PylRS:$tRNA^{Pyl}$ complex (green, PDB: 2ZNI).

Acknowledgments

We thank Kelly Sheppard and Noah Reynolds for materials and discussion. This work was supported by grants from the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy (DE-FG02-98ER20311) for work on PylRS, the National Institute of General Medical Sciences (GM22854), and by the National Science Foundation (MCB-0950474).

References

[1] Ibba, M. and Söll, D. (2000) Aminoacyl-tRNA synthesis. Annu. Rev. Biochem. 69, 617–650.
[2] Dale, T. and Uhlenbeck, O.C. (2005) Amino acid specificity in translation. Trends Biochem. Sci. 30, 659–665.
[3] Steitz, T.A. (2008) A structural understanding of the dynamic ribosome machine. Nat. Rev. Mol. Cell Biol. 9, 242–253.
[4] Sprinzl, M. (2006) Chemistry of aminoacylation and peptide bond formation on the 3′ terminus of tRNA. J. Biosci. 31, 489–496.
[5] Eriani, G., Delarue, M., Poch, O., Gangloff, J. and Moras, D. (1990) Partition of tRNA synthetases into two classes based on mutually exclusive sets of sequence motifs. Nature 347, 203–206.
[6] Sprinzl, M. and Cramer, F. (1975) Site of aminoacylation of tRNAs from *Escherichia coli* with respect to the 2′- or 3′-hydroxyl group of the terminal adenosine. Proc. Natl. Acad. Sci. USA 72, 3049–3053.
[7] Chinault, A.C., Tan, K.H., Hassur, S.M. and Hecht, S.M. (1977) Initial position of aminoacylation of individual *Escherichia coli*, yeast, and calf liver transfer RNAs. Biochemistry 16, 766–776.
[8] Griffin, B.E., Jarman, M., Reese, C.B., Sulston, J.E. and Trentham, D.R. (1966) Some observations relationg to acyl mobility in aminoacyl soluble ribonucleic acids. Biochemistry 5, 3638–3649.
[9] Weinger, J.S. and Strobel, S.A. (2006) Participation of the tRNA A76 hydroxyl groups throughout translation. Biochemistry 45, 5939–5948.
[10] von der, H.F. and Cramer, F. (1975) Isoleucyl-tRNA synthetase from baker's yeast: the 3′-hydroxyl group of the 3′-terminal ribose is essential for preventing misacylation of $tRNA^{Ile}$(CCA) with misactivated valine. FEBS Lett. 56, 215–217.
[11] Nordin, B.E. and Schimmel, P. (2002) Plasticity of recognition of the 3′-end of mischarged tRNA by class I aminoacyl-tRNA synthetases. J. Biol. Chem. 277, 20510–20517.
[12] Ling, J., Roy, H. and Ibba, M. (2007) Mechanism of tRNA-dependent editing in translational quality control. Proc. Natl. Acad. Sci. USA 104, 72–77.
[13] So, B.R., An, S., Kumar, S., Das, M., Turner, D.A., Hadad, C.M. and Musier-Forsyth, K. (2011) Substrate-mediated fidelity mechanism ensures accurate decoding of proline codons. J. Biol. Chem. 286, 31810–31820.
[14] Sauerwald, A., Zhu, W., Major, T.A., Roy, H., Palioura, S., Jahn, D., Whitman, W.B., Yates 3rd, J.R., Ibba, M. and Söll, D. (2005) RNA-dependent cysteine biosynthesis in archaea. Science 307, 1969–1972.
[15] Kamtekar, S., Hohn, M.J., Park, H.S., Schnitzbauer, M., Sauerwald, A., Söll, D. and Steitz, T.A. (2007) Toward understanding phosphoseryl-$tRNA^{Cys}$ formation: the crystal structure of *Methanococcus maripaludis* phosphoseryl-tRNA synthetase. Proc. Natl. Acad. Sci. USA 104, 2620–2625.
[16] Polycarpo, C., Ambrogelly, A., Berube, A., Winbush, S.M., McCloskey, J.A., Crain, P.F., Wood, J.L. and Söll, D. (2004) An aminoacyl-tRNA synthetase that specifically activates pyrrolysine. Proc. Natl. Acad. Sci. USA 101, 12450–12454.
[17] Kavran, J.M., Gundllapalli, S., O'Donoghue, P., Englert, M., Söll, D. and Steitz, T.A. (2007) Structure of pyrrolysyl-tRNA synthetase, an archaeal enzyme for genetic code innovation. Proc. Natl. Acad. Sci. USA 104, 11268–11273.
[18] Goldgur, Y., Mosyak, L., Reshetnikova, L., Ankilova, V., Lavrik, O., Khodyreva, S. and Safro, M. (1997) The crystal structure of phenylalanyl-tRNA synthetase from *Thermus thermophilus* complexed with cognate $tRNA^{Phe}$. Structure 5, 59–68.
[19] Roy, H., Ling, J., Alfonzo, J. and Ibba, M. (2005) Loss of editing activity during the evolution of mitochondrial phenylalanyl-tRNA synthetase. J. Biol. Chem. 280, 38186–38192.
[20] Wang, Z.U., Wang, Y.S., Pai, P.J., Russell, W.K., Russell, D.H. and Liu, W.R. (2012) A facile method to synthesize histones with posttranslational modification mimics. Biochemistry 51, 5232–5234.
[21] Davis, L. and Chin, J.W. (2012) Designer proteins: applications of genetic code expansion in cell biology. Nat. Rev. Mol. Cell Biol. 13, 168–182.
[22] Liu, C.C. and Schultz, P.G. (2010) Adding new chemistries to the genetic code. Annu. Rev. Biochem. 79, 413–444.
[23] Park, H.S., Hohn, M.J., Umehara, T., Guo, L.T., Osborne, E.M., Benner, J., Noren, C.J., Rinehart, J. and Söll, D. (2011) Expanding the genetic code of *Escherichia coli* with phosphoserine. Science 333, 1151–1154.
[24] Sampson, J.R. and Uhlenbeck, O.C. (1988) Biochemical and physical characterization of an unmodified yeast phenylalanine transfer RNA transcribed in vitro. Proc. Natl. Acad. Sci. USA 85, 1033–1037.
[25] Hohn, M.J., Park, H.S., O'Donoghue, P., Schnitzbauer, M. and Söll, D. (2006) Emergence of the universal genetic code imprinted in an RNA record. Proc. Natl. Acad. Sci. USA 103, 18095–18100.
[26] Andreeva, A., Howorth, D., Chandonia, J.M., Brenner, S.E., Hubbard, T.J., Chothia, C. and Murzin, A.G. (2008) Data growth and its impact on the SCOP database: new developments. Nucleic Acids Res. 36, D419–425.
[27] Roberts, E., Eargle, J., Wright, D. and Luthey-Schulten, Z. (2006) MultiSeq: unifying sequence and structure data for evolutionary analysis. BMC Bioinf. 7, 382.
[28] O'Donoghue, P. and Luthey-Schulten, Z. (2003) On the evolution of structure in aminoacyl-tRNA synthetases. Microbiol. Mol. Biol. Rev. 67, 550–573.
[29] Felsenstein, J. (1989) Mathematics vs. evolution: mathematical evolutionary theory. Science 246, 941–942.
[30] Safro, M. and Klipcan, L. (2013) The mechanistic and evolutionary aspects of the 2′- and 3′-OH paradigm in biosynthetic machinery. Biol. Direct 8, 17.
[31] Moor, N., Kotik-Kogan, O., Tworowski, D., Sukhanova, M. and Safro, M. (2006) The crystal structure of the ternary complex of phenylalanyl–tRNA synthetase with tRNAPhe and a phenylalanyl-adenylate analogue reveals a conformational switch of the CCA end. Biochemistry 45, 10572–10583.
[32] Fukunaga, R. and Yokoyama, S. (2007) Structural insights into the first step of RNA-dependent cysteine biosynthesis in archaea. Nat. Struct. Mol. Biol. 14, 272–279.

[33] Nozawa, K., O'Donoghue, P., Gundllapalli, S., Araiso, Y., Ishitani, R., Umehara, T., Söll, D. and Nureki, O. (2009) Pyrrolysyl-tRNA synthetase-tRNA^{Pyl} structure reveals the molecular basis of orthogonality. Nature 457, 1163–1167.
[34] Yanagisawa, T., Ishii, R., Fukunaga, R., Kobayashi, T., Sakamoto, K. and Yokoyama, S. (2008) Crystallographic studies on multiple conformational states of active-site loops in pyrrolysyl-tRNA synthetase. J. Mol. Biol. 378, 634–652.
[35] Ling, J., Reynolds, N. and Ibba, M. (2009) Aminoacyl-tRNA synthesis and translational quality control. Annu. Rev. Microbiol. 63, 61–78.
[36] Martinis, S.A. and Boniecki, M.T. (2010) The balance between pre- and post-transfer editing in tRNA synthetases. FEBS Lett. 584, 455–459.
[37] Roy, H., Ling, J., Irnov, M. and Ibba, M. (2004) Post-transfer editing in vitro and in vivo by the beta subunit of phenylalanyl-tRNA synthetase. EMBO J. 23, 4639–4648.
[38] Lincecum Jr., T.L., Tukalo, M., Yaremchuk, A., Mursinna, R.S., Williams, A.M., Sproat, B.S., Van Den, E.W., Link, A., Van Calenbergh, S., Grotli, M., Martinis, S.A. and Cusack, S. (2003) Structural and mechanistic basis of pre- and posttransfer editing by leucyl-tRNA synthetase. Mol. Cell 11, 951–963.